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Tseng et al.

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[54] **PLA_2 INHIBITORY COMPOUNDS**

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[52] **U.S. Cl.** **514/17**; 514/11; 530/317; 530/329; 530/330

[58] **Field of Search** 530/317, 329, 530/330; 514/11, 17

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[57] **ABSTRACT**

The present invention provides peptides and compounds which inhibit the enzyme activity of Type II phospholipases A_2. The preferred compounds are pentapeptides. Where the phospholipase is human Type II phospholipase A_2 the preferred peptides are FLSYK and KFLSY.

9 Claims, 7 Drawing Sheets

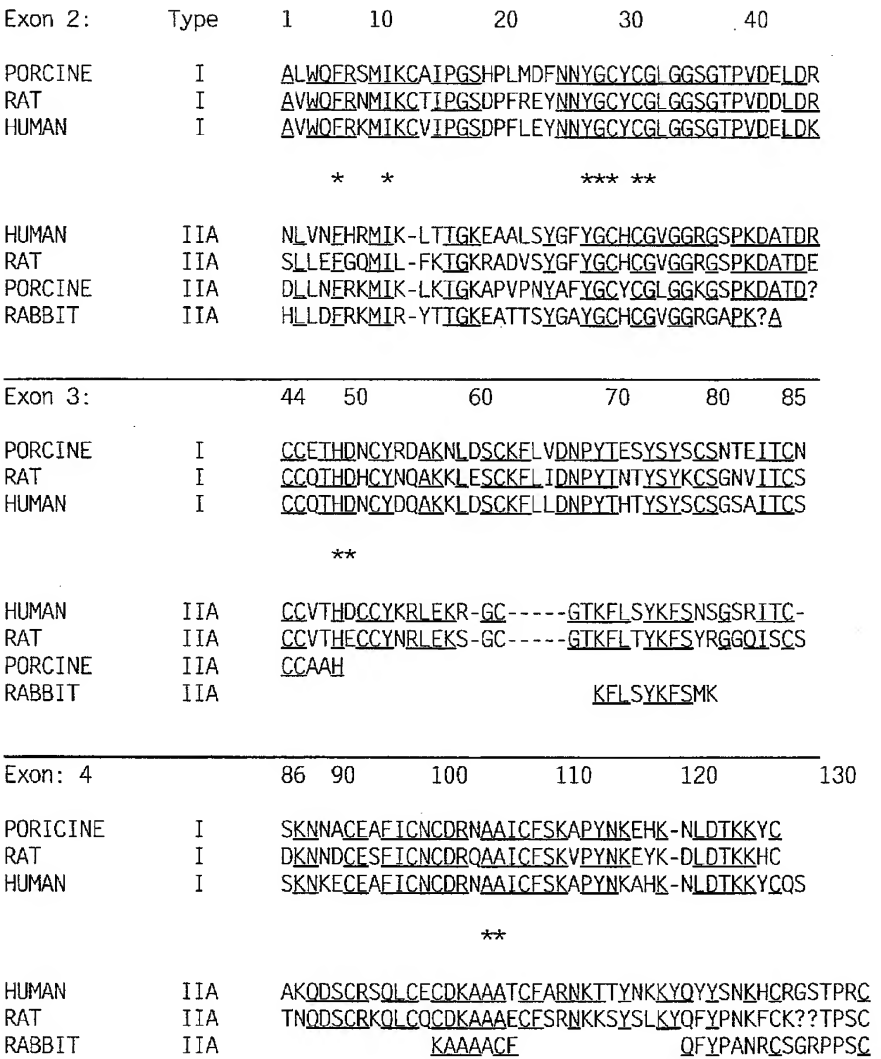
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FIG. 1

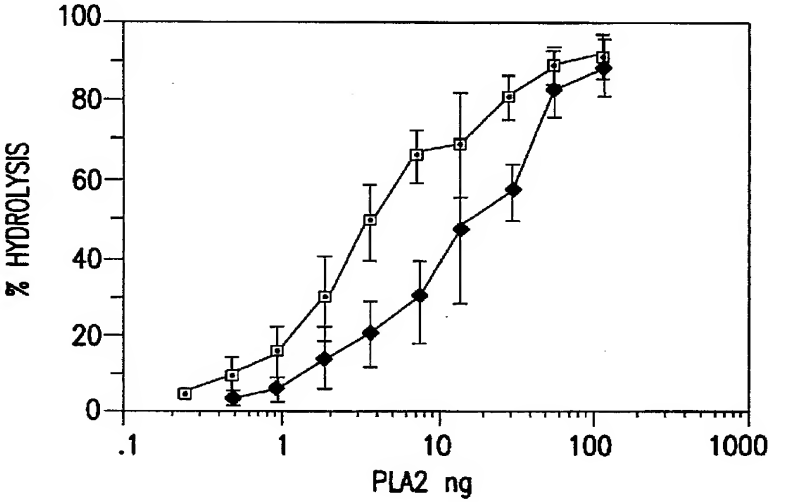
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FIG.2a

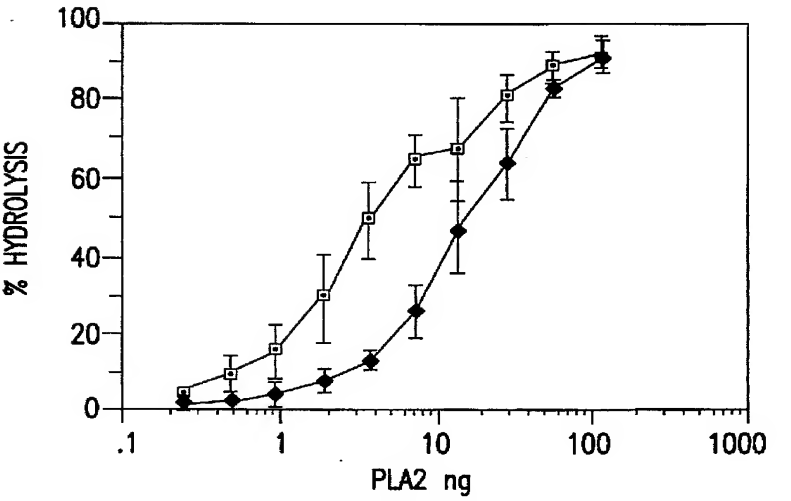
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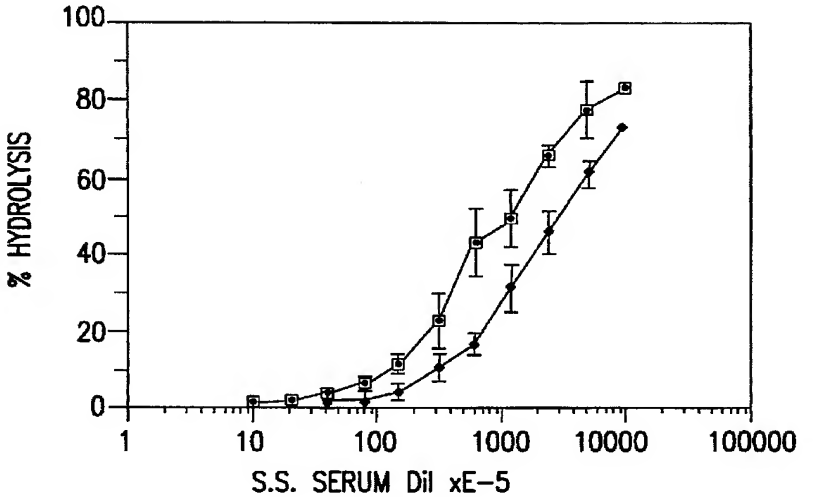
FIG.2b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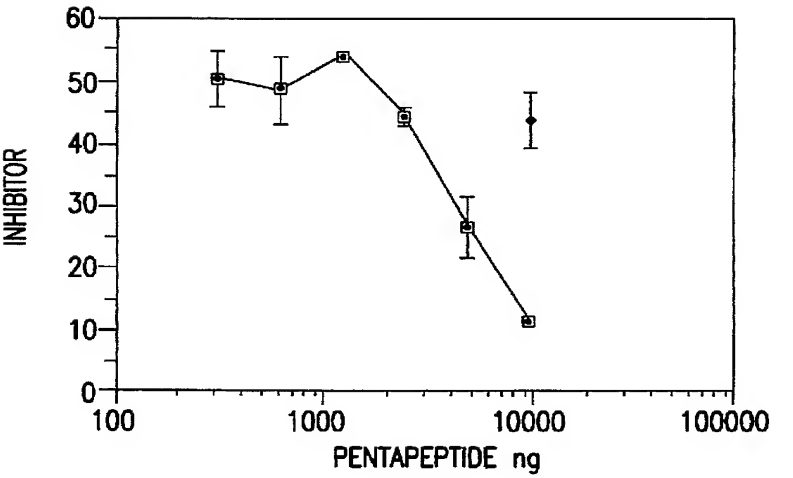
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FIG.3a

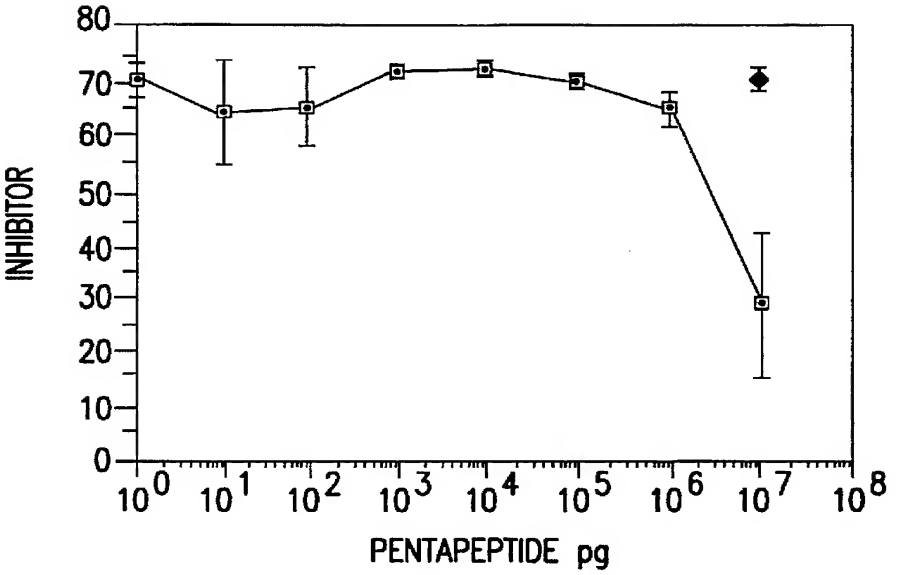
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FIG.3b

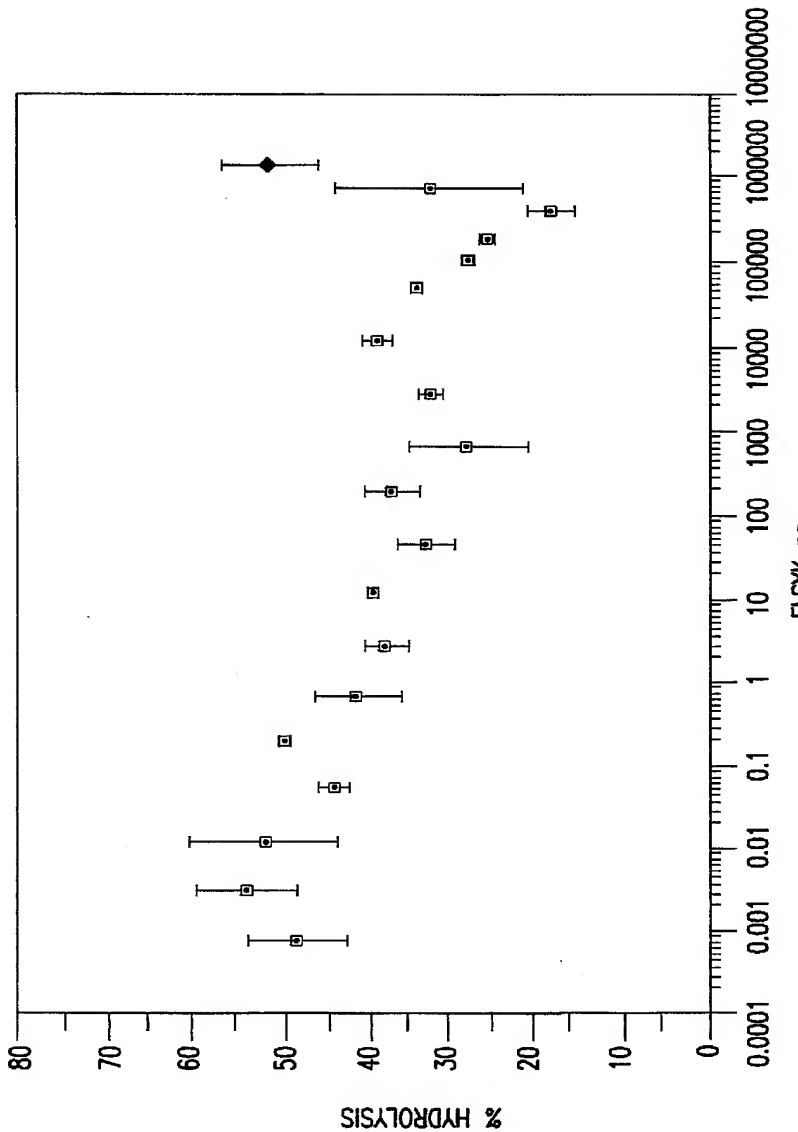
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FIG.4a

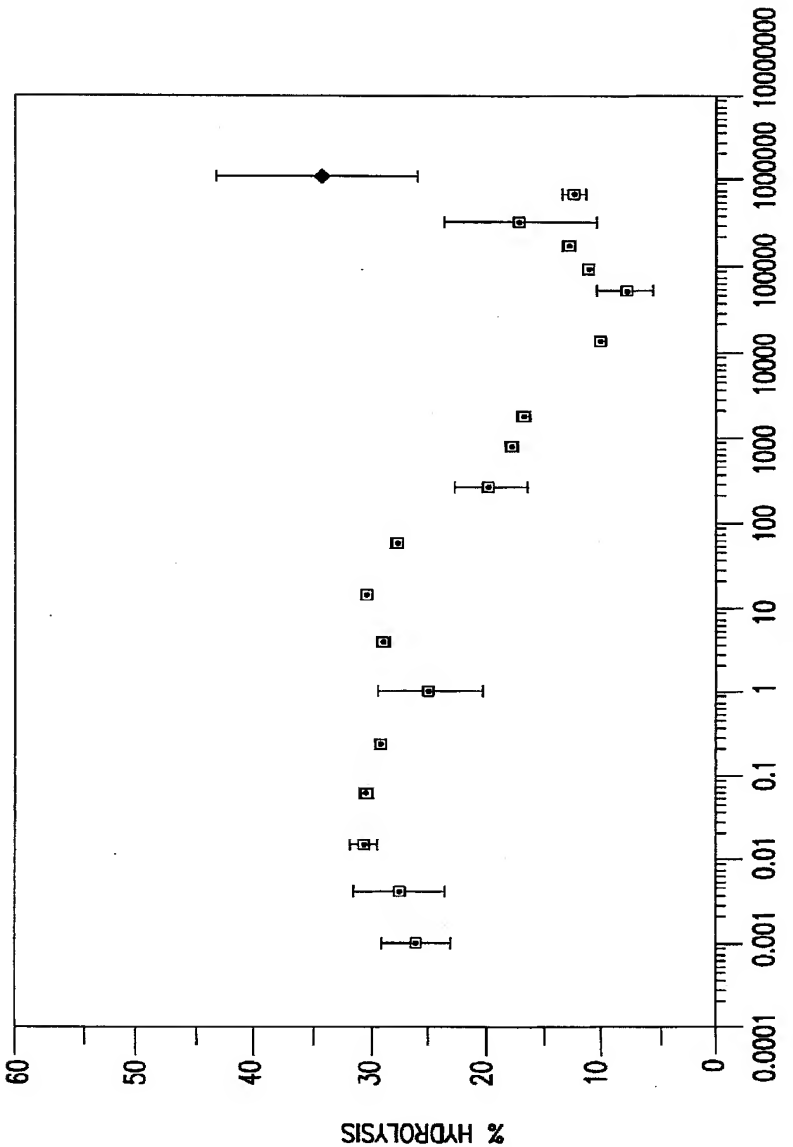
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FIG.4b

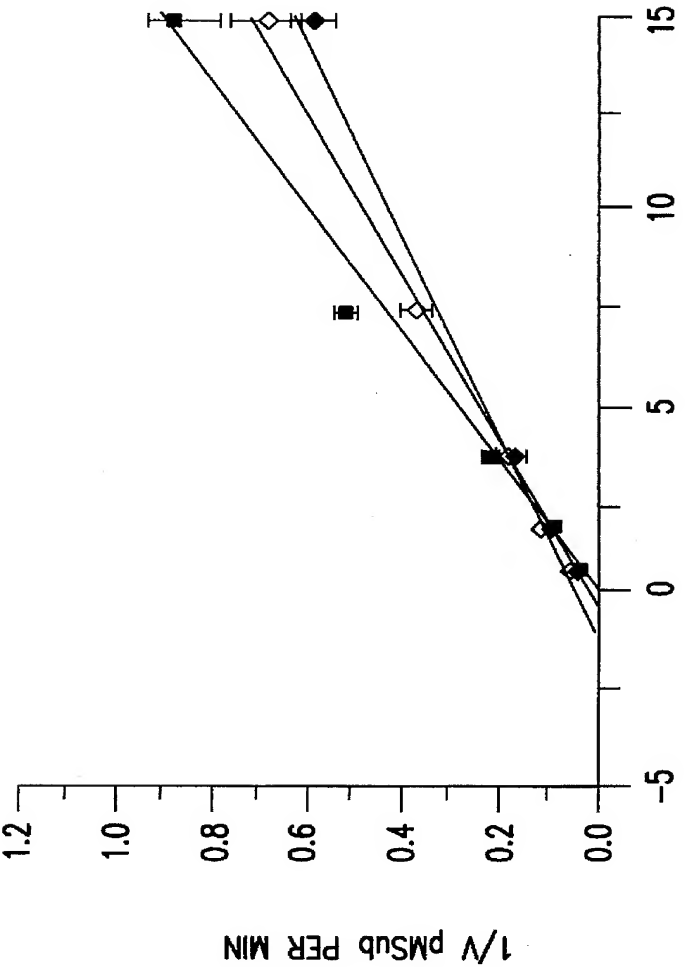

FIG.5

PLA_2 INHIBITORY COMPOUNDS

FIELD OF THE INVENTION

The present invention relates to peptides which inhibit the enzymatic activity of phospholipases A_2 (PLA_2s) and illustrated with peptides which inhibit the activity of Type II PLA_2's particularly synovial PLA_2 and snake PLA_2 (*Crotalus durissus* and *Crotalus atrox*). In addition, the present invention relates to pharmaceutical composition including, as the active ingredient these peptides and to methods of treatment involving the administration of this composition.

BACKGROUND OF THE INVENTION

Phospholipases A_2 constitute a diverse family of enzymes with two subclasses (Type I and Type II) (FIG. 1), based on the positions of the disulphide bonds in the molecules while bee venom PLA_2 constitutes a third substantially distinct class of PLA_2. X-ray crystallography has revealed that the segments comprising the functional substructure of the enzyme is similar in classes. This similarity is particularly striking when the structurally-related Type I/II enzymes are compared with bee venom enzyme (2). PLA_2 hydrolyses the sn-2 acyl ester bond of phosphoglycerides initiating the release of fatty acid precursors of inflammatory eicosanoids. Human synovial PLA_2 (a Type II molecule) has recently been isolated and identified (3). The same PLA 2 has been implicated in the pathogensis of several inflammatory discases in humans such as rheumatoid arthritis and Gram negative septic shock (7;8).

Murine, inhibitory monoclonal antibodies raised against synovial PLA_2 have demonstrated pre-clinical efficacy. Accordingly, there is interest in the development of compositions which inhibit the enzymatic activity of PLA_2.

Tryptic digestion of human synovial PLA_2 and subsequent separation and analysis of the fragments by EPLC gave a very interesting and unexpected result for one of the peaks in that it contained two peptides; one a heptapeptide (the N-terminal peptide) and the other a pentapeptide, FLSYK (SEQ ID NO:8) (corresponding to residues 70–74 in other PLA_2 molecules, based on three-dimensional structural "homology" of mammalian PLA_2 amino acid sequences (1,4)). The pentapeptide was found in an earlier eluting, fully resolved peak (as expected). Since the HPLC system failed to fully resolve these two peptides in the latter peak, these data suggest that the two peptides had a strong affinity for one another and raised questions as to the structural implications of this. X-ray diffraction studies (5,6) have shown that amino acid residues in the two peptides are close to the active site of the enzyme and are important in forming or stablising the channel in which the 1,2-diacyl-3-sn-phosphoglyceride substrate is precisely positioned for hydrolysis of the 2-ester bond. The first turn of the N-terminal helix (residues 1 to 12) is stablised by a hydrogen bond network provided by the N-terminus and residue 4, elements of residues 69 to 71 and a water mediated link to the catalytic residues; residues 2 and 5 form the "floor" of the channel, residue 9 forms the right wall and the left wall is formed by residue 69 (either tyrosine or lysine usually) which is predicted to move after the substrate has docked and to form a hydrogen bond with the sn-3 phosphate of the substrate. The chemical evidence of the strong interactions between the heptapeptide and the pentapeptide prompted the supposition that the PLA_2 activity may be inhibited in the presence of either one of these peptides.

Using synthetic peptide chemistry the present inventors have prepared the pentapeptide FLSYK and demonstrated

that addition of it to the assay medium decreased the enzyme activity of human synovial PLA_2 (FIG. 2*a*). Furthermore, it has been demonstrated that the pentapeptide that occupies the 70–74 region of snake PLA_2 (WDIYR) also inhibited the activity of snake PLA_2 (see FIG. 3*b*). It is believed that this inhibition is mediated by the peptide binding to the amino terminal end of the enzyme and blocking the reaction either by blocking the substrate access to the hydrophobic channel or by distorting the structure sufficiently to prevent correct orientation of the substrate.

SUMMARY OF THE INVENTION

Accordingly, in a first aspect the present invention consists in a linear or cyclic peptide of at least 5 residues which inhibits the enzymatic activity of human synovial PLA_2, the peptide having the following formula:

$$A_1\text{-}A_2\text{-}A_3\text{-}A_4\text{-}A_5\text{-}A_6\text{-}A_7$$

in which

A_1 is hydrogen or one or two naturally occurring amino acids

A_2 is F or Y or W or absent

A_3 is L or V or I or M

A_4 is S or T

A_5 is Y or F or W

A_6 is K or R or H or absent

A_7 is OH or one or two naturally occurring amino acids.

In a preferred embodiment the peptide is a pentapeptide.

In another preferred embodiment of the present invention A_1 is H and A_7 is OH.

In a further preferred embodiment of the present invention the peptide is FLSYK (SEQ ID NO:8) or KFLSY (SEQ ID NO:9) and most preferably FLSYK.

In a second aspect the present invention consists in a linear or cyclic peptide of at least 5 residues which inhibits the enzymatic activity of *Crotalus durissus* PLA_2, the peptide having the following formula:

$$B_1\text{-}B_2\text{-}B_3\text{-}B_4\text{-}B_5\text{-}B_6\text{-}B_7$$

in which

B_1 is hydrogen or one or two naturally occurring amino acids

B_2 is W or F or Y or absent

B_3 is D or E

B_4 is I or V or L or M

B_5 is Y or F or W

B_6 is R or K or H or absent

B_7 is OH or one or two naturally occurring amino acids.

In a preferred embodiment the peptide is a pentapeptide.

In another preferred embodiment of the present invention B_1 is H and B_7 is OH.

In a further preferred embodiment of the present invention the peptide is WDIYR (SEQ ID NO:10).

In a third aspect the present invention consists in a linear or cyclic peptide of at least 5 residues which inhibits the enzymatic activity of *Crotalus atrox* PLA_2, the peptide having the following formula:

$$C_1\text{-}C_2\text{-}C_3\text{-}C_4\text{-}C_5\text{-}C_6\text{-}C_7$$

in which

C_1 is hydrogen or one or two naturally occurring amino acids

C_2 is T or S or absent

C_3 is V or I or L or M

C_4 is S or T

C_5 is Y or F or W

C_6 is T or S or absent

C_7 is OH or one or two naturally occurring amino acids.

In a preferred embodiment the peptide is a pentapeptide.

In another preferred embodiment of this aspect of the present invention C_1 is H and C_7 is OH.

In a further preferred embodiment of this aspect of the present invention the peptide is TVSYT (SEQ ID NO:11).

As will be clear to those skilled in the art from the disclosure provided herein, the peptides of the first and second aspect of the present invention illustrate how the enzymatic activity of other PLA_2s may be inhibited. This inhibition may be achieved by compounds which interact with the N-terminal amino acid sequence of the PLA_2 molecule in a manner such that the channel into which the phospholipid diffuses prior to catalytic cleavage is destabilized.

Accordingly, in a fourth aspect the present invention consists in a compound which inhibits the enzymatic activity of phospholipase A_2, the compound being characterized in that it interacts with the N-terminal amino acid sequence of the phospholipase A_2 such that the channel into which the phospholipid diffuses prior to catalytic cleavage is either blocked or destabilized.

In a preferred embodiment of the present invention the PLA_2 is human PLA_2 and the compound is a peptide.

In a preferred embodiment of the present invention the peptide has the amino acid sequence FLSYK or KFLSY.

As will be clear to those skilled in the art, the present inventors have found that the enzymatic activity of a phospholipase A_2 can be inhibited by a peptide having a sequence corresponding to a sequence selected from the region of residues 69 to 75 of the phospholipase 2.

Accordingly, in a fifth aspect the present invention consists in a peptide of 5 or 6 residues which inhibits the enzymatic activity of a phospholipase A_2, the peptide having an amino acid sequence corresponding to a sequence selected from the region of residues 69–75 of the phospholipase A_2.

In a preferred embodiment this aspect of the present invention the peptide is a pentapeptide and has an amino acid sequence corresponding to the sequence from residue 69–73 or 70–74 of the phospholipase A_2.

In a further preferred embodiment of the present invention the phospholipase A_2 is human phospholipase A_2.

In a sixth aspect the present invention consists in a composition for use in treating a subject suffering from septic shock rheumatoid arthritis and/or other inflammatory diseases, the composition comprising a therapeutically acceptable amount of peptide or compound of the first, fourth or fifth aspect of the present invention and a pharmaceutical acceptable sterile carrier.

In a seventh aspect the present invention consists in a method of treating septic shock and/or inflammatory disease in a subject comprising administering to the subject the composition of the sixth aspect of the present invention.

It will be appreciated by those skilled in the art that a number of modifications may be made to the peptides of the present invention without deleteriously effecting the biological activity of the peptide. This may be achieved by various changes, such as insertions, deletions and substitutions, either conservative or non-conservative in the peptide sequence where such changes do not substantially decrease the biological activity of the peptide. By conservative substitutions the intended combinations are:

G, A; V, I, L, M; D, E; N, Q; S, T; K, R, H; and F, Y, W.

It may also be possible to add various groups to the peptide of the present invention to confer advantages such as increased potency or extended half life in vivo, without substantially decreasing the biological activity of the peptide.

It is intended that such modifications to the peptide of the present invention which do not result in a decrease in biological activity are with in the scope of the present invention.

DETAILED DESCRIPTION OF THE PRESENT INVENTION

In order that the nature of the present invention may be more clearly understood, preferred forms thereof will now be described with reference to the following examples and Figures, in which:

FIG. 1 shows mammalian PLA_2 amino acid sequences (SEQ ID NOS. 1, 2, 3, 4, 5, 6 and 7).

FIG. 2: Inhibition of human PLA_2 using the peptide FLSYK.

FIG. 2(*a*) was obtained using a peptide from a tryptic digest of the enzyme (n=7 ⊡ control ♦ inhibitor), 2(*b*) and 2(*c*) with a synthetic peptide n=11 ⊡ control ♦ inhibitor ⊡ control ♦ inhibitor, respectively. The synthetic peptide also inhibits the enzyme in septic shock serum [FIG. 2(*c*)].

FIG. 3: Dose response curves showing increasing inhibitor with increasing amount of FLSYK and human recombinant Type II PLA_2 (3*a* ⊡ inhibitor ♦ control) and in PLA_2 in septic shock serum (3*b* ⊡ inhibitor ♦ control).

FIG. 4: Dose response curves for FLSYK (4*a* ⊡ PLA_2 ♦ control) and WDIYR (4*b* ⊡ snake (II) ♦ control) on human PLA_2 and snake (Crotalus Durissus) PLA_2 respectively. Both peptides occupy similar sites in their parent proteins and show inhibitory properties for the enzymatic activity.

FIG. 5 shows a Lineweaver-Buspe plot showing inhibition of PLA_2 by FLSYK (PLA_2 ♦ 10 ug ■ FLSYK, ◇ 1 ug FLSYK).

Inhibition of PLA2 Activity

Proteins and Peptides

1. Synovial PLA_2, snake PLA_2 (Crotalus Durissus and Crotalus ATR?)
2. Phe-Leu-Ser-Tyr-Lys (FLSYK) (SEQ ID NO:8)
3. Acetyl-Phe-Leu-Ser-Tyr-Lys-Methyl ester (Ac-FLSYK-OMe)
4. Trp-Asp-Ile-Tyr-Arg (WDIYR) (SEQ ID NO:10)
5. Lys-Phe-Leu-Ser-Tyr (KFLSY) (SEQ ID NO:9)
6. Thr-Val-Ser-Tyr-Thr (TVSYT) (SEQ ID NO:12)
7. Phe-Lys-Thr-Tyr-Ser (FKTYS) (SEQ ID NO:13)
8. Thr-Glu-Ser-Tyr-Ser (TESYS) (SEQ ID NO:14)
9. Gly-Thr-Lys-Phe-Leu-Ser-Tyr-Lys-Phe-Ser-Asn (GTKFLSYKFSN) (SEQ ID NO:15)
10. Lys-Phe-Leu-Ser-Tyr-Tyr (KFLSYY) (SEQ ID NO:16)
11. Phe-Leu-Ser-Tyr (FLSY) (SEQ ID NO:17)
12. Phe-Leu-Ser-Tyr-Lys-NH_2. (FLSYK-NH_2)

Tryptic Digestion of PLA2:

Approximately 100 µg of PLA 2 was dissolved in 300 µl of 1 MTris pH 8.0 15 µl of Trypsin solution (10µ/1M Tris pH

8) was added and the peptide/trypsin solution was incubated for 2 hours at 37° C. 5 µl of neat TFA was used to lower the. pH to terminate the digestion. The whole solution was subjected to microbore HPLC fractionation.

Microbore HPLC fractionation:

An ABI Microbore syringe pump system Model 140 was used. Detector wavelength was set at 220 nm at 0.5 aufs. A RP-300 1×100 mm was used. Fractionation was carried out by running a linear buffer gradient from 0.1% TFA in water to 0.1% TFA, 70% acetonitrile in water over sixty minutes. Amino acid sequences identified from fractions were:

Fraction #2 (K)YQYYSNK

Fraction #4 FLSYK

Fraction #5 FLSYK NLVNFHR

Fraction #7* EALLSYGFYG(C)H(C)GVGGR (C)(C) VTHD(C)(C)YK SQL(C)E(C)DK IT(C)AK AAAT(C) FAR

* peptides are held together by cystinyl bonds; ( ) denotes tentative assignment.

Fraction #9 EAALSYGFYG

Peptide Synthesis:

Peptide synthesis was carried out in an ABI Peptide Synthesiser Model 430A. T-Boc chemistry was used. HF cleavage was used to release peptide from the solid support.

PLA2 Serial Dilution:

Control: 10 µl of a standard PLA_2 solution was used at a concentration of 120 ng/10 µl in 20 mM Tris pH 8. Serial dilution was done by adding 20 mM Tris pH 8 buffer to the final volume of 20 µl.

Inhibitor solution: Pentapeptide was usually dissolved in 1 µl of 0.1% TFA solution and further 9 µl of 20 mM Tris pH8 was added. This solution was always maintained around pH7–8. 10 µl of this inhibitor solution was added into 10 µl of PLA_2 solution. Incubation: all samples were incubated at 37° C. for one hour.

PLA_2 solution: A standard PLA 2 solution was prepared in 20 mM Tris pH8.0 so that 10 µl will give 50% (approx) hydrolysis.

Pentapeptide solution: A standard pentapeptide solution was made to 10 mg/ml in 0.1% TFA. 100 µl was taken out and neutralised with 900 µl 20 mM Tris pH8. 10 µl (10 µg was taken out for dose response together with 10 µl of the PLA_2 solution). Serial dilution was carried out on 10 µl aliquots with 20 mM Tris pH 8.

Septic shock experiments:

Septic shock serum was diluted 1/100 for dose response experiments and used neat for serial dilution. Final reaction volume was always in the ratio of 10 µl serum/10 µl Tris or pentapeptide solution.

Activity assay:

PLA_2 activity was measured using a mixed micelle phosphatidylethanolamine (PE)/sodium deoxycholate assay, modified from a method described by Seilhamer et al (1). The PE substrate was prepared by dissolving freshly desiccated PE (Amersham, Bucks, England) in 2% DOC, then diluting this to 0.22 nmoles PE and 0.04% DOC per sample in assay buffer (50 mM Tris-HCl, pH 8.5, 2 mM calcium chloride, 150 mM sodium chloride, 0.04% DOC). The sample was prepared by mixing 10 µl of the test material with 10 µl mM Tris-HCl pH7.4 and leaving at 37° C. for 10 minutes. The reaction was started by the addition of 25 µl prewarmed substrate and terminated by addition of 10 µl 100 mM EDTA. The reaction mixture (30 µl was spotted and dried on silica TLC plates (Merck, Darmstadt, West Germany), and the plates were chromatographed using chloroform:methanol:acetic acid (90:10:1) as solvent. The dried plates were exposed overnight with Kodak X OMAT AR film. Radioactivity at the origin and of the liberated arachidonic acid was counted and the percent hydrolysis by PLA 2 determined.

A summary of the results obtained with peptides corresponding to residues 70–7u of several Type I and Type II enymes are set out in Table 1. These results show that there is considerable species specificity in that peptides active against one species of PLA_2 were not active against the other species tested. In addition none of the peptides tested were active against PLA_2 type 1. This result indicates that inhibition by peptides from this region of PLA_2 (70–74) appears to occur only on type II enymes.

Peptide 5 was shown to be an active inhibitor of human Type II PLA_2, however peptides 7, 8, 9, 10, 11 and 12 were all formed to be negative. This suggests that the peptide must be of a certain size to show inhibition and that inhibition will occur only with the specific sequence desired from the specific Type II enyme being tested.

TABLE 1

Type Enzyme Inhibitor	II Syno PLA_2	II Crot.Dur. PLA_2	II Crot.Atr. PLA_2	I N.N.Atra PLA_2	I Por.Pan PLA_2
$sPLA_2$ (FLSYK)	+	–	–	–	–
Crot.Dur (WDIYR)	–	+	–	–	–
Crot.Atr (TVSYT)	–	–	+	–	–
N.N.At (FKTYS)	–	–	–	–	–
Por.Pan (TESYS)	–	–	–	–	–

$sPLA_2$- Human Type II PLA_2
Crot.Dur- Crotalus decrissurs PLA_2
Crot.Atr- Crotalus atrox PLA_2
N.N.AT- Naja naja atrox PLA_2
Por.Pan.- Porcine pancreatic PLA_2

From the above results the present inventors believe that short peptides from the 70–74th region will most likely compete with the substrate for access to the active site and give inhibitory effects. It is believed that variation of the length of the peptides present in these regions may result in a optimisation of the inhibition.

The pentapeptide apparently possesses helical structure (approximately one and a half turns). Since the helical structures are stablised by hydrogen bonds between the C=O of one residue and NH of the fourth residue along the chain, the structure of the pentapeptide may be somewhat unstable and be more sensitive to the environment than a longer helical molecule. On the other hand, it would be expected that the range of sizes that is effective will be limited because of the limited access to the active site of PLA_2.

It is believed that the usual interchange of a hydrophobic residue e.g. Leu to Ile, Ser to Thr could also maintain the inhibitory effect. That is, amino acid residues alike in either charge or hydrophobicity could possibly be interchanged with those in the models without destroying the specific interaction of the two regions. Since helix-helix interactions are possibly the cause of the inhibitory action. small increases in the length of the peptides could stablise this structure.

The results obtained in these studies also suggest that monoclonal antibodies could be raised against epitopes containing one or both of the peptide regions to effect a similar result on the PLA_2 activity. Such monoclonal antibodies could be produced using standard techniques well known in the art.

As will be apparent to those skilled in the art the principle of the present invention will also have application for the inhibition of the activity of enzymes other than PLA_2 eg. the neuraminadase enzyme of the influenza virus or neuropeptide Y. It is envisaged that as biological active proteins in general, possess an active conformation which is stabilized by interaction with the surrounding chain of amino acids, that peptides adjacent to, and capable of interaction with the residues within the active site will inhibit the activity of the enzyme. It is intended that such other peptides are included within the scope of the present invention.

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

REFERENCES

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i i i) NUMBER OF SEQUENCES: 17

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 124 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(i i i) HYPOTHETICAL: NO

(i v) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Ala	Leu	Trp	Gln	Phe	Arg	Ser	Met	Ile	Lys	Cys	Ala	Ile	Pro	Gly	Ser
1				5					10					15	
His	Pro	Leu	Met	Asp	Phe	Asn	Asn	Tyr	Gly	Cys	Tyr	Cys	Gly	Leu	Gly
			20					25					30		
Gly	Ser	Gly	Thr	Pro	Val	Asp	Glu	Leu	Asp	Arg	Cys	Cys	Glu	Thr	His
		35					40					45			
Asp	Asn	Cys	Tyr	Arg	Asp	Ala	Lys	Asn	Leu	Asp	Ser	Cys	Lys	Phe	Leu
	50					55					60				
Val	Asp	Asn	Pro	Tyr	Thr	Glu	Ser	Tyr	Ser	Tyr	Ser	Cys	Ser	Asn	Thr
65					70					75					80
Glu	Ile	Thr	Cys	Asn	Ser	Lys	Asn	Asn	Ala	Cys	Glu	Ala	Phe	Ile	Cys
				85					90					95	
Asn	Cys	Asp	Arg	Asn	Ala	Ala	Ile	Cys	Phe	Ser	Lys	Ala	Pro	Tyr	Asn
			100					105					110		
Lys	Glu	His	Lys	Asn	Leu	Asp	Thr	Lys	Lys	Tyr	Cys				
		115					120								

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 124 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

-continued

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Ala Val Trp Gln Phe Arg Asn Met Ile Lys Cys Thr Ile Pro Gly Ser
 1               5                  10                  15

Asp Pro Phe Arg Glu Tyr Asn Asn Tyr Gly Cys Tyr Cys Gly Leu Gly
            20                  25                  30

Gly Ser Gly Thr Pro Val Asp Asp Leu Asp Arg Cys Cys Gln Thr His
        35                  40                  45

Asp His Cys Tyr Asn Gln Ala Lys Lys Leu Glu Ser Cys Lys Phe Leu
    50                  55                  60

Ile Asp Asn Pro Tyr Thr Asn Thr Tyr Ser Tyr Lys Cys Ser Gly Asn
65                  70                  75                  80

Val Ile Thr Cys Ser Asp Lys Asn Asn Asp Cys Glu Ser Phe Ile Cys
                85                  90                  95

Asn Cys Asp Arg Gln Ala Ala Ile Cys Phe Ser Lys Val Pro Tyr Asn
            100                 105                 110

Lys Glu Tyr Lys Asp Leu Asp Thr Lys Lys His Cys
        115                 120
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 126 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
Ala Val Trp Gln Phe Arg Lys Met Ile Lys Cys Val Ile Pro Gly Ser
 1               5                  10                  15

Asp Pro Phe Leu Glu Tyr Asn Asn Tyr Gly Cys Tyr Cys Gly Leu Gly
            20                  25                  30

Gly Ser Gly Thr Pro Val Asp Glu Leu Asp Lys Cys Cys Gln Thr His
        35                  40                  45

Asp Asn Cys Tyr Asp Gln Ala Lys Lys Leu Asp Ser Cys Lys Phe Leu
    50                  55                  60

Leu Asp Asn Pro Tyr Thr His Thr Tyr Ser Tyr Ser Cys Ser Gly Ser
65                  70                  75                  80

Ala Ile Thr Cys Ser Ser Lys Asn Lys Glu Cys Glu Ala Phe Ile Cys
                85                  90                  95

Asn Cys Asp Arg Asn Ala Ala Ile Cys Phe Ser Lys Ala Pro Tyr Asn
            100                 105                 110

Lys Ala His Lys Asn Leu Asp Thr Lys Lys Tyr Cys Gln Ser
        115                 120                 125
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

-continued

(A) LENGTH: 124 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(i i i) HYPOTHETICAL: NO

(i v) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Asn Leu Val Asn Phe His Arg Met Ile Lys Leu Thr Thr Gly Lys Glu
1 5 10 15

Ala Ala Leu Ser Tyr Gly Phe Tyr Gly Cys His Cys Gly Val Gly Gly
20 25 30

Arg Gly Ser Pro Lys Asp Ala Thr Asp Arg Cys Cys Val Thr His Asp
35 40 45

Cys Cys Tyr Lys Arg Leu Glu Lys Arg Gly Cys Gly Thr Lys Phe Leu
50 55 60

Ser Tyr Lys Phe Ser Asn Ser Gly Ser Arg Ile Thr Cys Ala Lys Gln
65 70 75 80

Asp Ser Cys Arg Ser Gln Leu Cys Glu Cys Asp Lys Ala Ala Ala Thr
85 90 95

Cys Phe Ala Arg Asn Lys Thr Thr Tyr Asn Lys Lys Tyr Gln Tyr Tyr
100 105 110

Ser Asn Lys His Cys Arg Gly Ser Thr Pro Arg Cys
115 120

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 125 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(i i i) HYPOTHETICAL: NO

(i v) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Ser Leu Leu Glu Phe Gly Gln Met Ile Leu Phe Lys Thr Gly Lys Arg
1 5 10 15

Ala Asp Val Ser Tyr Gly Phe Tyr Gly Cys His Cys Gly Val Gly Gly
20 25 30

Arg Gly Ser Pro Lys Asp Ala Thr Asp Glu Cys Cys Val Thr His Glu
35 40 45

Cys Cys Tyr Asn Arg Leu Glu Lys Ser Gly Cys Gly Thr Lys Phe Leu
50 55 60

Thr Tyr Lys Phe Ser Tyr Arg Gly Gly Gln Ile Ser Cys Ser Thr Asn
65 70 75 80

Gln Asp Ser Cys Arg Lys Gln Leu Cys Gln Cys Asp Lys Ala Ala Ala
85 90 95

Glu Cys Phe Ser Arg Asn Lys Lys Ser Tyr Ser Leu Lys Tyr Gln Phe
100 105 110

Tyr Pro Asn Lys Phe Cys Lys Xaa Xaa Thr Pro Ser Cys

-continued

115 120 125

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 47 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(i i i) HYPOTHETICAL: NO

(i v) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Asp Leu Leu Asn Phe Arg Lys Met Ile Lys Leu Lys Thr Gly Lys Ala
1 5 10 15

Pro Val Pro Asn Tyr Ala Phe Tyr Gly Cys Tyr Cys Gly Leu Gly Gly
20 25 30

Lys Gly Ser Pro Lys Asp Ala Thr Asp Xaa Cys Cys Ala Ala His
35 40 45

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 71 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: protein

(i i i) HYPOTHETICAL: NO

(i v) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:7:

His Leu Leu Asp Phe Arg Lys Met Ile Arg Tyr Thr Thr Gly Lys Glu
1 5 10 15

Ala Thr Thr Ser Tyr Gly Ala Tyr Gly Cys His Cys Gly Val Gly Gly
20 25 30

Arg Gly Ala Pro Lys Xaa Ala Lys Phe Leu Ser Tyr Lys Phe Ser Met
35 40 45

Lys Lys Ala Ala Ala Ala Cys Phe Gln Phe Tyr Pro Ala Asn Arg Cys
50 55 60

Ser Gly Arg Pro Pro Ser Cys
65 70

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: both

(i i) MOLECULE TYPE: peptide

(i i i) HYPOTHETICAL: NO

(i v) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

-continued

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Phe Leu Ser Tyr Lys
1 5

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: both

(i i) MOLECULE TYPE: peptide

(i i i) HYPOTHETICAL: NO

(i v) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Lys Phe Leu Ser Tyr
1 5

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: both

(i i) MOLECULE TYPE: peptide

(i i i) HYPOTHETICAL: NO

(i v) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Trp Asp Ile Tyr Arg
1 5

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: both

(i i) MOLECULE TYPE: peptide

(i i i) HYPOTHETICAL: NO

(i v) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Thr Val Ser Tyr Thr
1 5

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: both

-continued

(i i) MOLECULE TYPE: peptide

(i i i) HYPOTHETICAL: NO

(i v) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Thr Val Ser Thr Thr
1 5

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: both

(i i) MOLECULE TYPE: peptide

(i i i) HYPOTHETICAL: NO

(i v) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Phe Lys Thr Tyr Ser
1 5

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: both

(i i) MOLECULE TYPE: peptide

(i i i) HYPOTHETICAL: NO

(i v) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Thr Glu Ser Tyr Ser
1 5

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: both

(i i) MOLECULE TYPE: peptide

(i i i) HYPOTHETICAL: NO

(i v) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Gly Thr Lys Phe Leu Ser Tyr Lys Phe Ser Asn
1 5 10

-continued

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: both

(i i) MOLECULE TYPE: peptide

(i i i) HYPOTHETICAL: NO

(i v) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Lys Phe Leu Ser Tyr Tyr
1 5

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: both

(i i) MOLECULE TYPE: peptide

(i i i) HYPOTHETICAL: NO

(i v) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Phe Leu Ser Tyr
1

We claim:

1. A linear or cyclic peptide of at least 5 residues which inhibits the enzymatic activity of human synovial PLA_2, the peptide having the following formula:

$$A_1\text{-}A_2\text{-}A_3\text{-}A_4\text{-}A_5\text{-}A_6$$

in which

A_1 is K or R or H or absent

A_2 is F or Y or W

A_3 is L or V or I or M

A_4 is S or T

A_5 is Y or F or W

A_6 is K or R or H or absent.

2. A peptide as claimed in claim 1 in which the peptide is FLSYK or KFLSY.

3. A peptide as claimed in claim 1 in which the phospholipase A_2 is human phospholipase A_2.

4. A composition for use in treating the subject suffering from rheumatoid arthritis, septic shock and/or inflammatory disease, the composition comprising a therapeutically effective amount of the peptide as claimed in claim 1 and a pharmaceutically acceptable sterile carrier.

5. A peptide as claimed in claim 1, in which either A_1 or A_6 is absent.

6. A linear peptide which inhibits the enzymatic activity of *Crotalus durissus* PLA_2, the peptide having the following formula:

$$B_2\text{-}B_3\text{-}B_4\text{-}B_5\text{-}B_6$$

in which

B_2 is W or F or Y

B_3 is D or E

B_4 is I or V or L or M

B_5 is Y or F or W

B_6 is R or K or H.

7. A peptide as claimed in claim 6 in which the peptide is WDIYR.

8. A linear peptide which inhibits the enzymatic activity of *Crotalus atrox* PLA_2, the peptide having the following formula:

$$C_2\text{-}C_3\text{-}C_4\text{-}C_5\text{-}C_6$$

in which

C_2 is T or S

C_3 is V or I or L or M

C_4 is T or S

C_5 is Y or F or W

C_6 is T or S.

9. A peptide as claimed in claim 8 in which the peptide is TVSYT.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 5,656,602

DATED : August 12, 1997

INVENTOR(S) : Albert Peng Sheng Tseng et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

On the title page, item [54] and column 1, line 1, the title should be --PLA2 INHIBITORY COMPOUNDS--.

In the Claims:

Col. 19, line 41 (claim 1), "or cyclic" should be deleted.

Signed and Sealed this

Fourteenth Day of April, 1998

Bruce Lehman

Attest:

Attesting Officer

BRUCE LEHMAN

Commissioner of Patents and Trademarks